How to use MSstats as an external tool in Skyline

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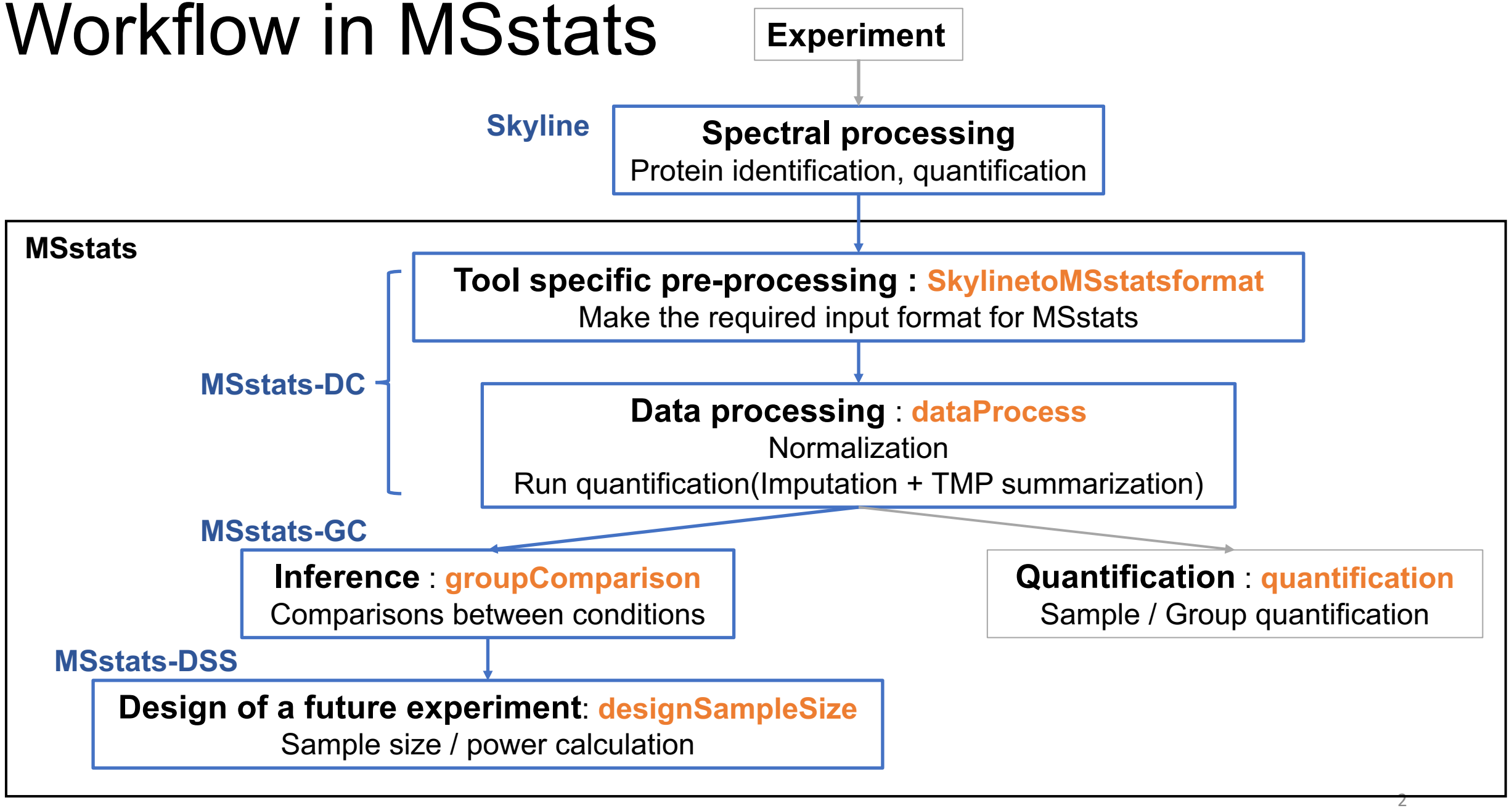
Two-day short course : Case studies in Quantitative Proteomics

at ASMS 2018

Take-home message

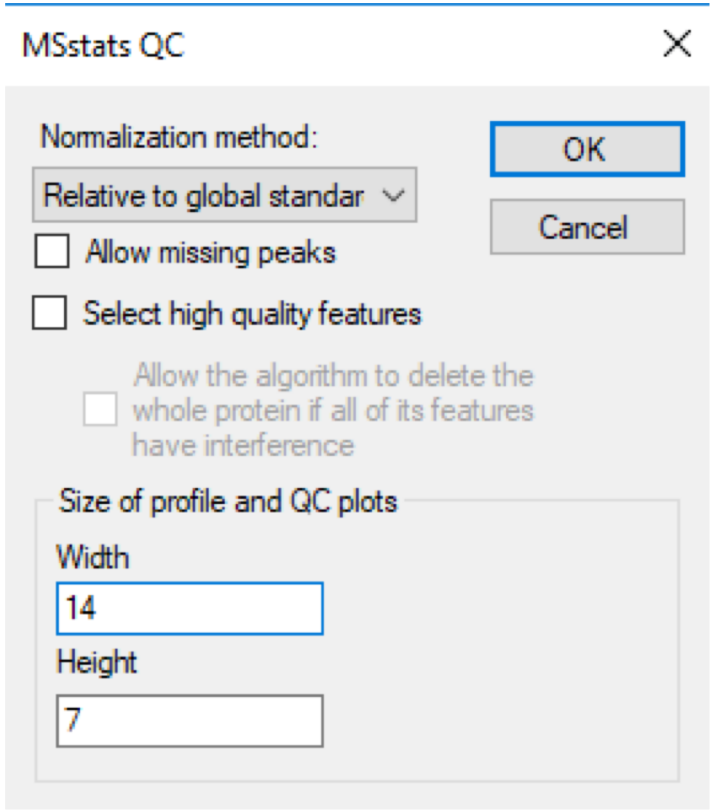
1. How to run MSstats external tool in Skyline
2. How to correctly annotate your experiments
3. Different parameter settings have different result
4. Other workflow with MSstats R package

Workflow in MSstats



1. How to run MSstats external tool in Skyline

Heart failure with rat,
Label-free SRM
‘Group studies’ in tutorial



- Tool > MSstats > data processing
- Normalization : ‘Relative to global standard’ with one peptide, VVD
- Data is processed for statistical analysis and three pdfs with visualization are generated in the folder with skyline file.
  - Profile plot
  - QC plot
  - Condition plot
- Video

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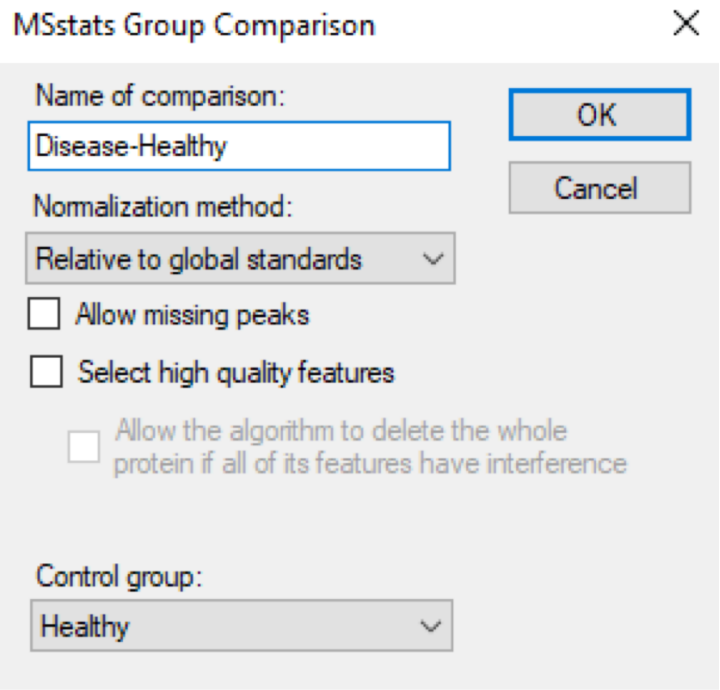

- Tool > MSstats > group comparison
- Normalization : ‘Relative to global standard’ with one peptide, VVD
- Set up which comparison you want.
- csv file with testing result and pdfs with visualization for results are generated in the folder with skyline file.
  - Volcano plot
  - Condition plot
- Video

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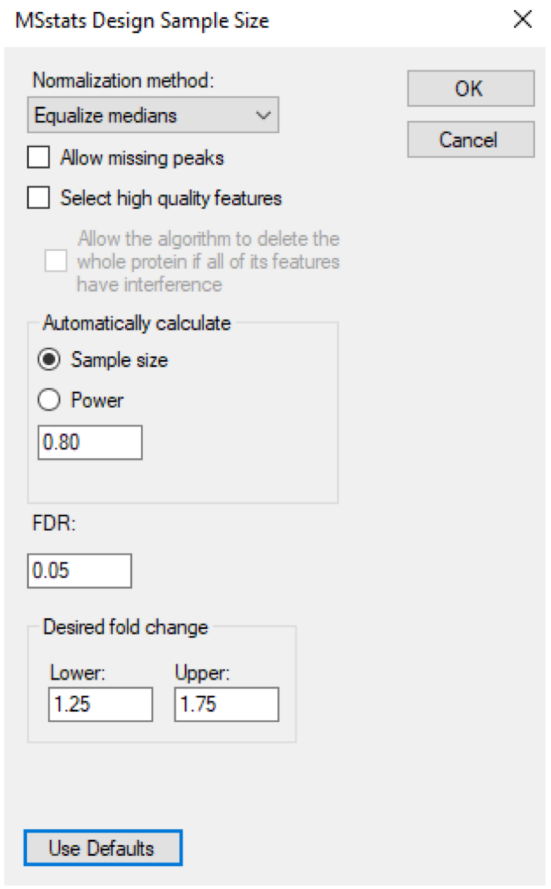
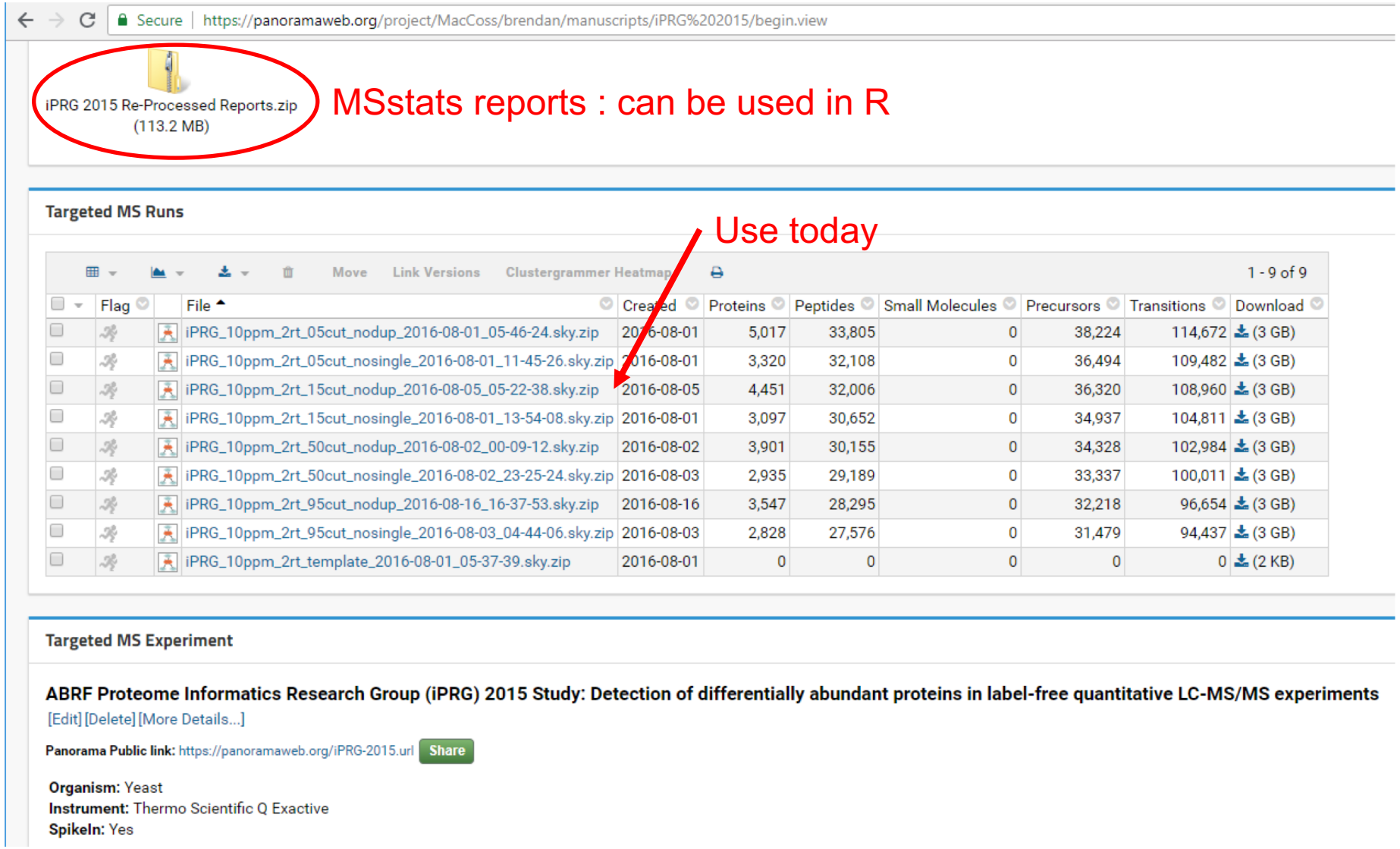
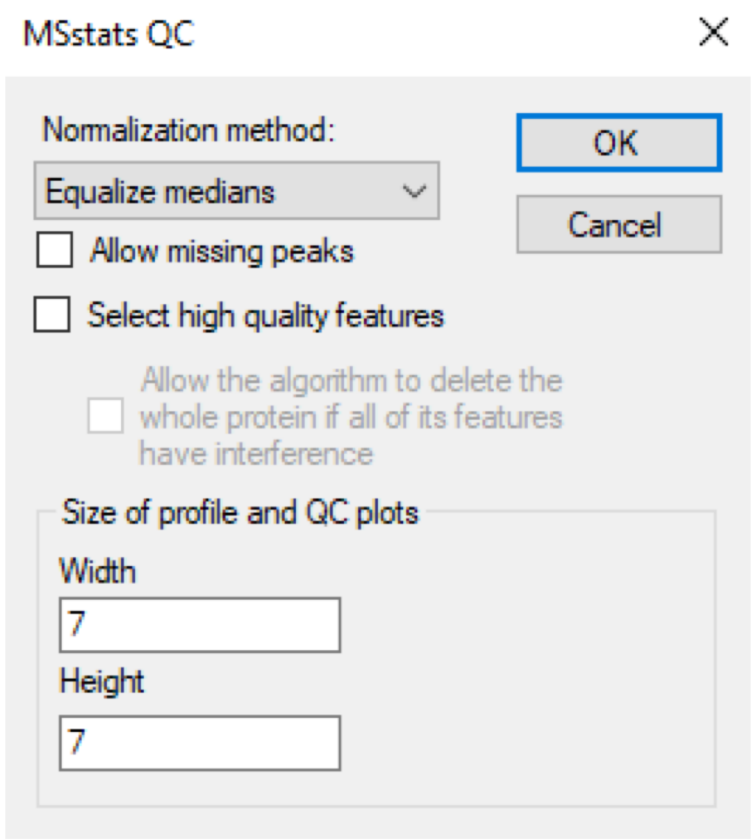


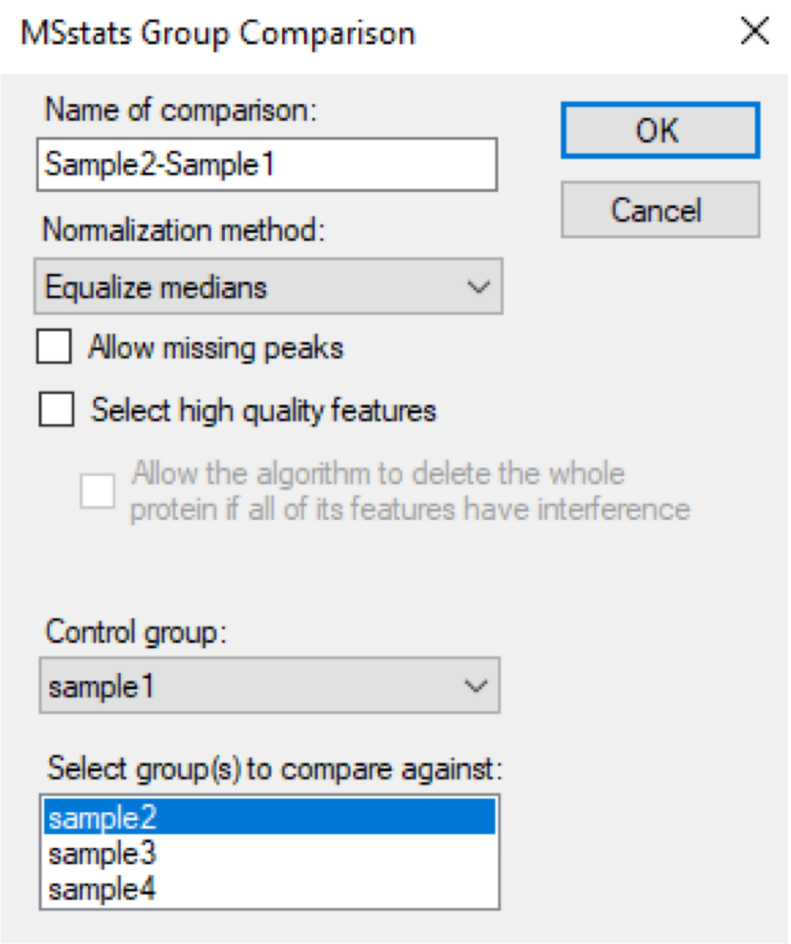
- Tool > MSstats > design sample size
- Normalization : 'Relative to global standard' with one peptide, VVD
- Change values as you want.
- csv file with sample size calculation or power and pdf for visualization is generated in the folder with skyline file.
- Video

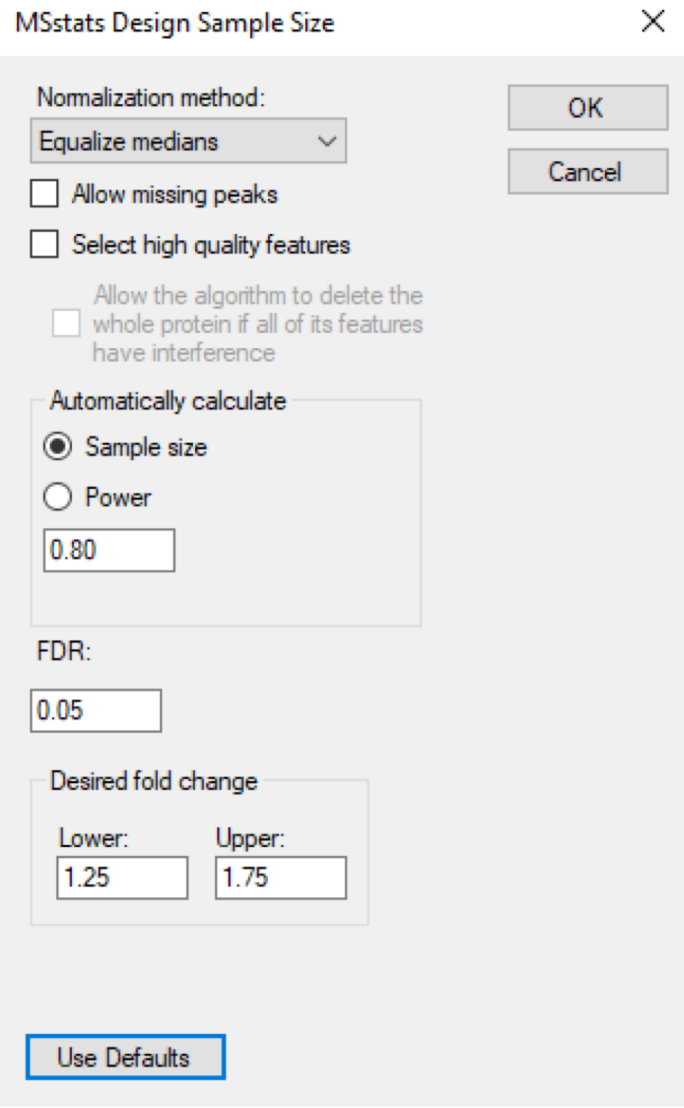
Statistical analysis for iPRG data with MSstats external tool in Skyline



Statistical analysis for iPRG data with MSstats external tool in Skyline



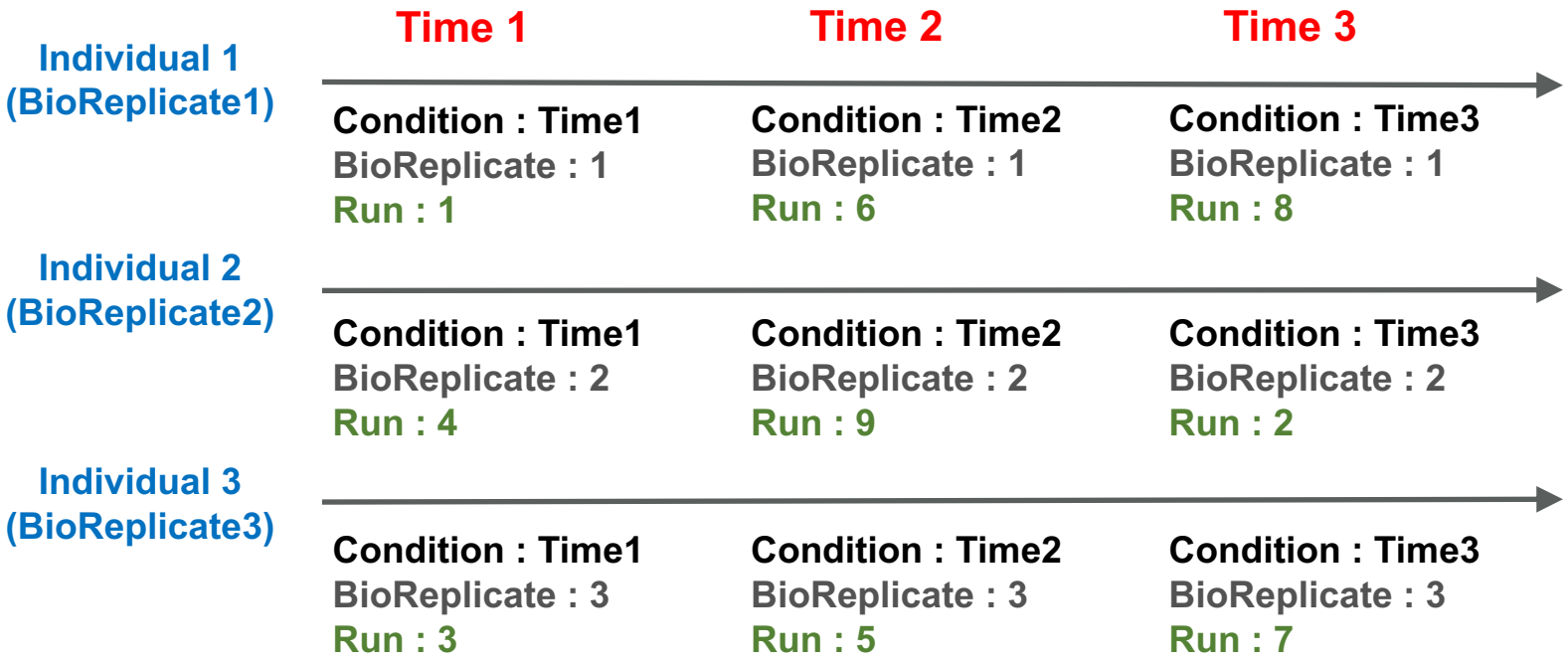




2. How to correctly annotate your experiments

- Time- course
- Paired-design
- Case-control

Time-course



Annotation

Condition	BioReplicate	Run
Time1	1	1
Time2	1	6
Time3	1	8
Time1	2	4
Time2	2	9
Time3	2	2
Time1	3	3
Time2	3	5
Time3	3	7

*The injection order (MS run) is randomized.

Paired-design

	Healthy tissue1	Healthy tissue2	Lung cancer
Individual 1 (BioReplicate1)	Condition : Healthy1 BioReplicate : 1 Run : 1	Condition : Healthy2 BioReplicate : 1 Run : 6	Condition : cancer BioReplicate : 1 Run : 8
Individual 2 (BioReplicate2)	Condition : Healthy1 BioReplicate : 2 Run : 4	Condition : Healthy2 BioReplicate : 2 Run : 9	Condition : cancer BioReplicate : 2 Run : 2
Individual 3 (BioReplicate3)	Condition : Healthy1 BioReplicate : 3 Run : 3	Condition : Healthy2 BioReplicate : 3 Run : 5	Condition : cancer BioReplicate : 3 Run : 7

*The injection order (MS run) is randomized.

Annotation

Condition	BioReplicate	Run
Healthy tissue1	1	1
Healthy tissue2	1	6
Lung cancer	1	8
Healthy tissue1	2	4
Healthy tissue2	2	9
Lung cancer	2	2
Healthy tissue1	3	3
Healthy tissue2	3	5
Lung cancer	3	7

Case-control

	Healthy group	Lung cancer
Individual 1 (BioReplicate1)	Condition : Healthy BioReplicate : 1 Run : 1	
Individual 2 (BioReplicate2)	Condition : Healthy BioReplicate : 2 Run : 4	
Individual 3 (BioReplicate3)	Condition : Healthy BioReplicate : 3 Run : 3	
Individual 4 (BioReplicate4)		Condition : cancer BioReplicate : 4 Run : 5
Individual 5 (BioReplicate5)		Condition : cancer BioReplicate : 5 Run : 6
Individual 6 (BioReplicate6)		Condition : cancer BioReplicate : 6 Run : 2

Annotation

Condition	BioReplicate	Run
Healthy	1	1
Healthy	2	4
Healthy	3	3
Lung cancer	4	5
Lung cancer	5	6
Lung cancer	6	2

*The injection order (MS run) is randomized.

Case-control with tech replicates

	Healthy group	Lung cancer
Individual 1 (BioReplicate1)	Condition : Healthy BioReplicate : 1 Run : 1, 8, 15	
Individual 2 (BioReplicate2)	Condition : Healthy BioReplicate : 2 Run : 4, 11, 14	
Individual 3 (BioReplicate3)	Condition : Healthy BioReplicate : 3 Run : 3, 10, 17	
Individual 4 (BioReplicate4)		Condition : cancer BioReplicate : 4 Run : 5, 9, 13
Individual 5 (BioReplicate5)		Condition : cancer BioReplicate : 5 Run : 6, 12, 16
Individual 6 (BioReplicate6)		Condition : cancer BioReplicate : 6 Run : 2, 7, 18

*The injection order (MS run) is randomized.

Annotation

Condition	BioReplicate	Run
Healthy	1	1
Healthy	1	8
Healthy	1	15
Healthy	2	4
Healthy	2	11
Healthy	2	14
Healthy	3	3
Healthy	3	10
Healthy	3	17
Lung cancer	4	5
Lung cancer	4	9
Lung cancer	4	13
Lung cancer	5	6
Lung cancer	5	12
Lung cancer	5	16
Lung cancer	6	2
Lung cancer	6	7
Lung cancer	6	18

Example with different annotation

iPRG2015

Case1 – correct for this experiment

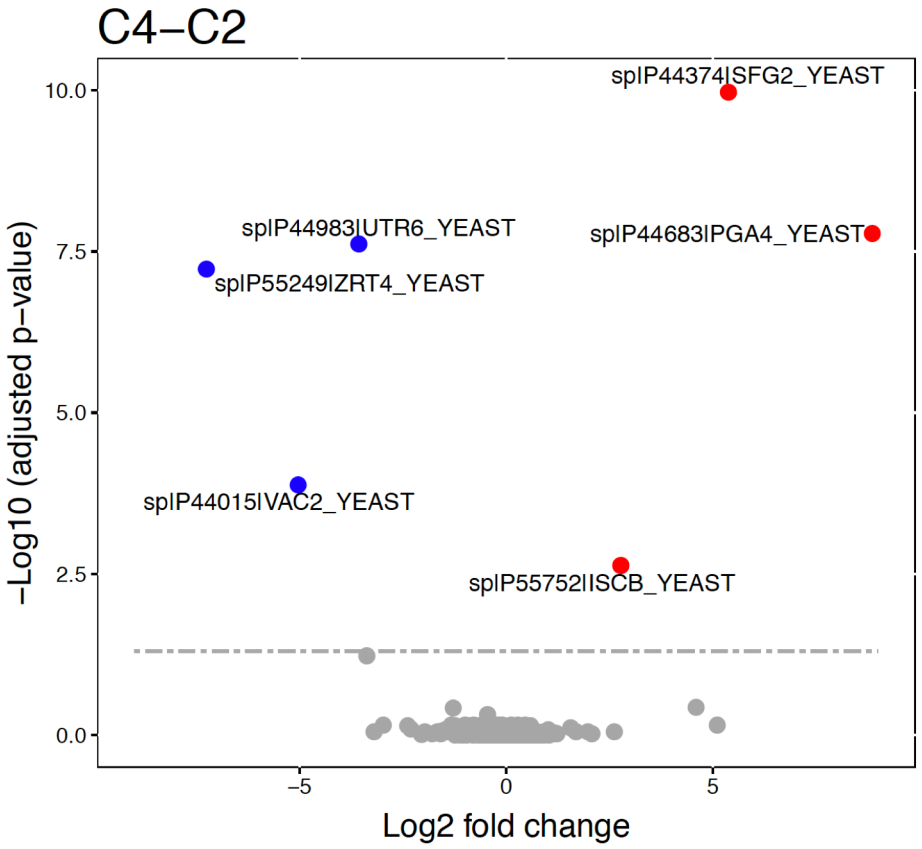
Run	Condition	BioReplicate
JD_06232014_sample1-A.raw	Condition1	1
JD_06232014_sample1_B.raw	Condition1	1
JD_06232014_sample1_C.raw	Condition1	1
JD_06232014_sample2_A.raw	Condition2	2
JD_06232014_sample2_B.raw	Condition2	2
JD_06232014_sample2_C.raw	Condition2	2
JD_06232014_sample3_A.raw	Condition3	3
JD_06232014_sample3_B.raw	Condition3	3
JD_06232014_sample3_C.raw	Condition3	3
JD_06232014_sample4_B.raw	Condition4	4
JD_06232014_sample4-A.raw	Condition4	4
JD_06232014_sample4_C.raw	Condition4	4

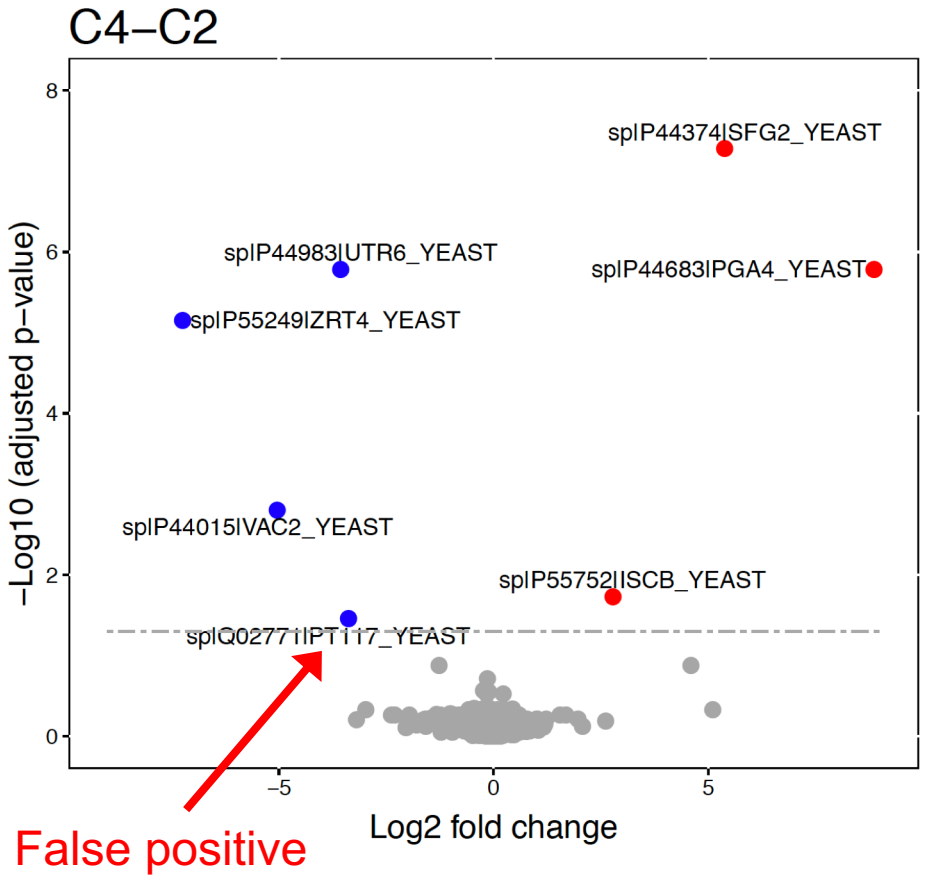
Case2 – incorrect for this experiment, common mistake

Run	Condition	BioReplicate
JD_06232014_sample1-A.raw	Condition1	1
JD_06232014_sample1_B.raw	Condition1	2
JD_06232014_sample1_C.raw	Condition1	3
JD_06232014_sample2_A.raw	Condition2	1
JD_06232014_sample2_B.raw	Condition2	2
JD_06232014_sample2_C.raw	Condition2	3
JD_06232014_sample3_A.raw	Condition3	1
JD_06232014_sample3_B.raw	Condition3	2
JD_06232014_sample3_C.raw	Condition3	3
JD_06232014_sample4_B.raw	Condition4	2
JD_06232014_sample4-A.raw	Condition4	1
JD_06232014_sample4_C.raw	Condition4	3

Example with different annotation

N of significant proteins	Case1-correct	Case2-incorrect
Background protein	1	4
Spike protein	28 out of 30	26 out of 30





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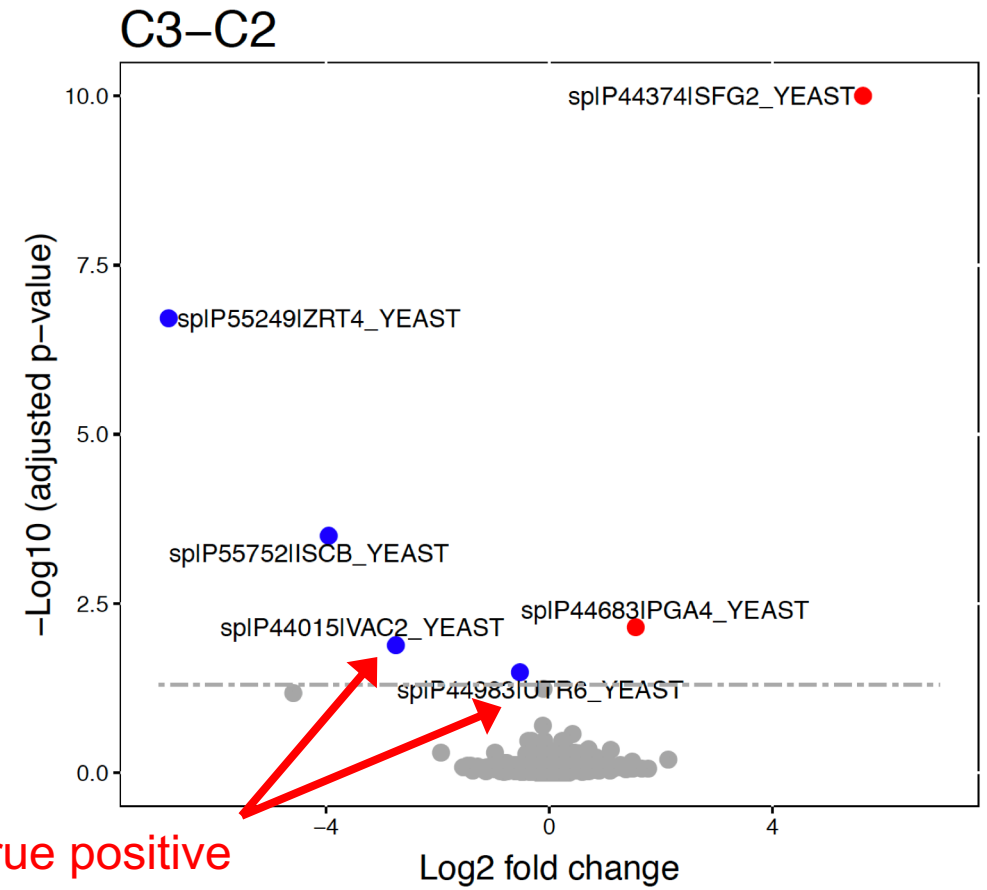
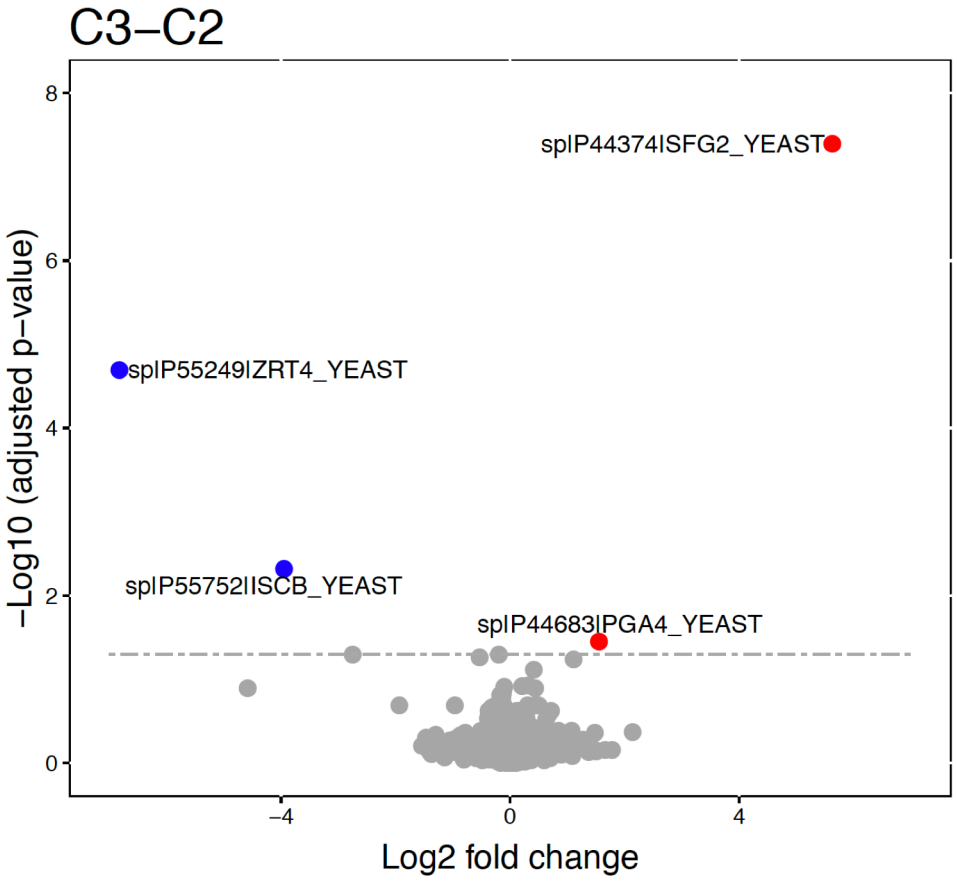
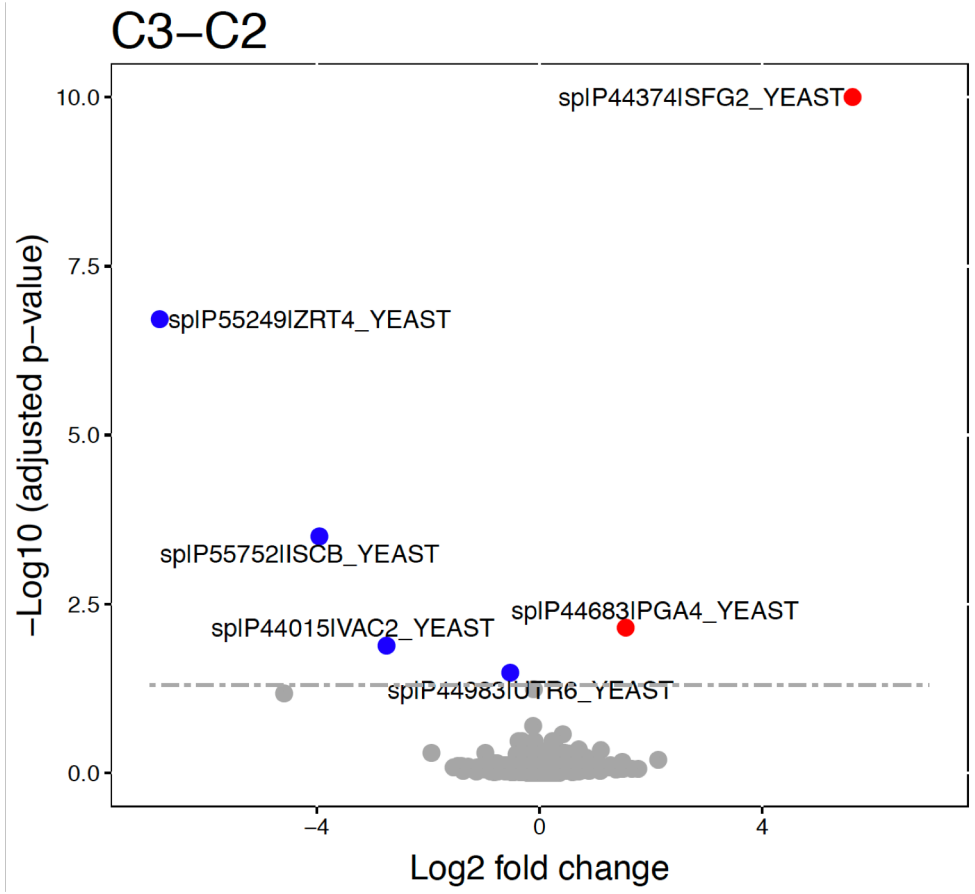


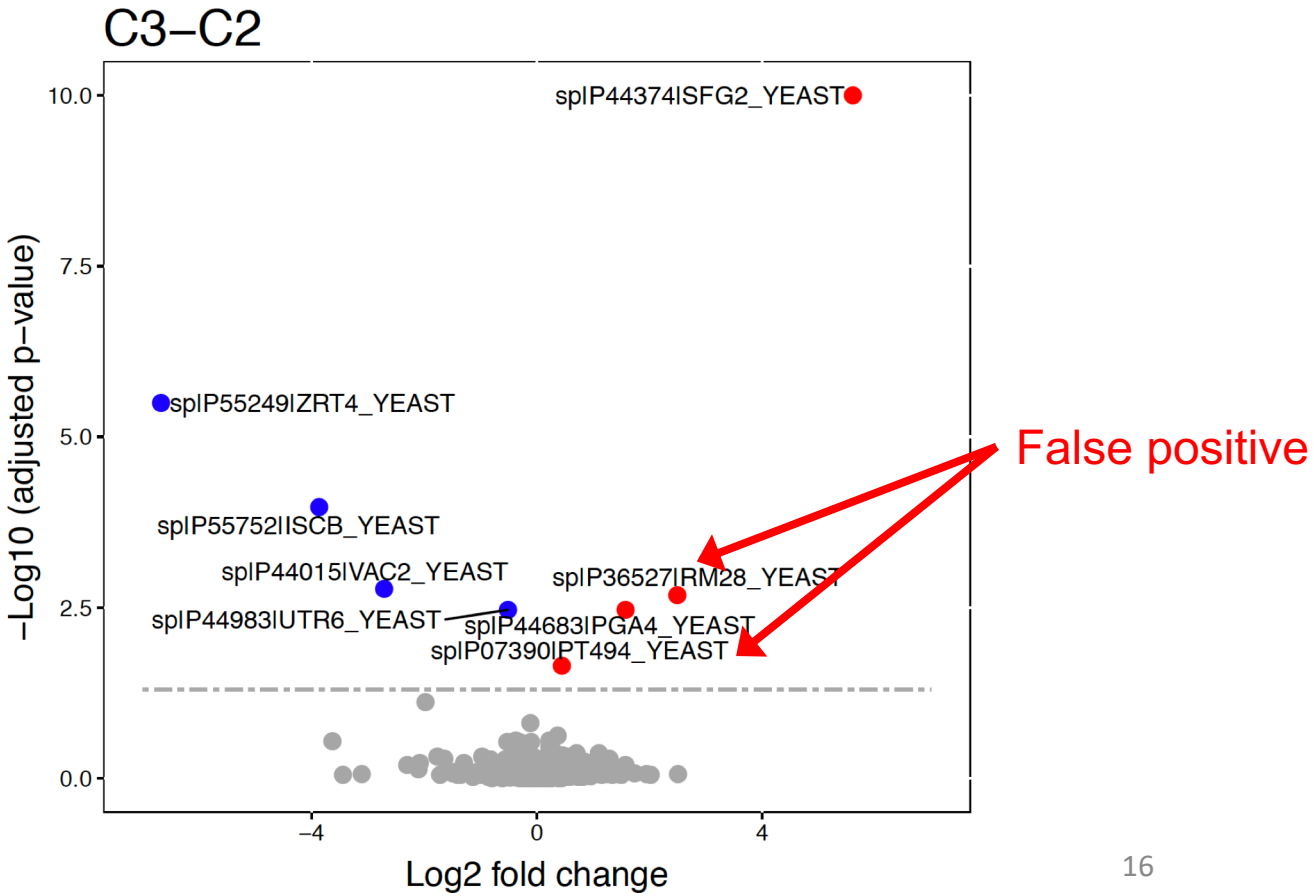
True positive



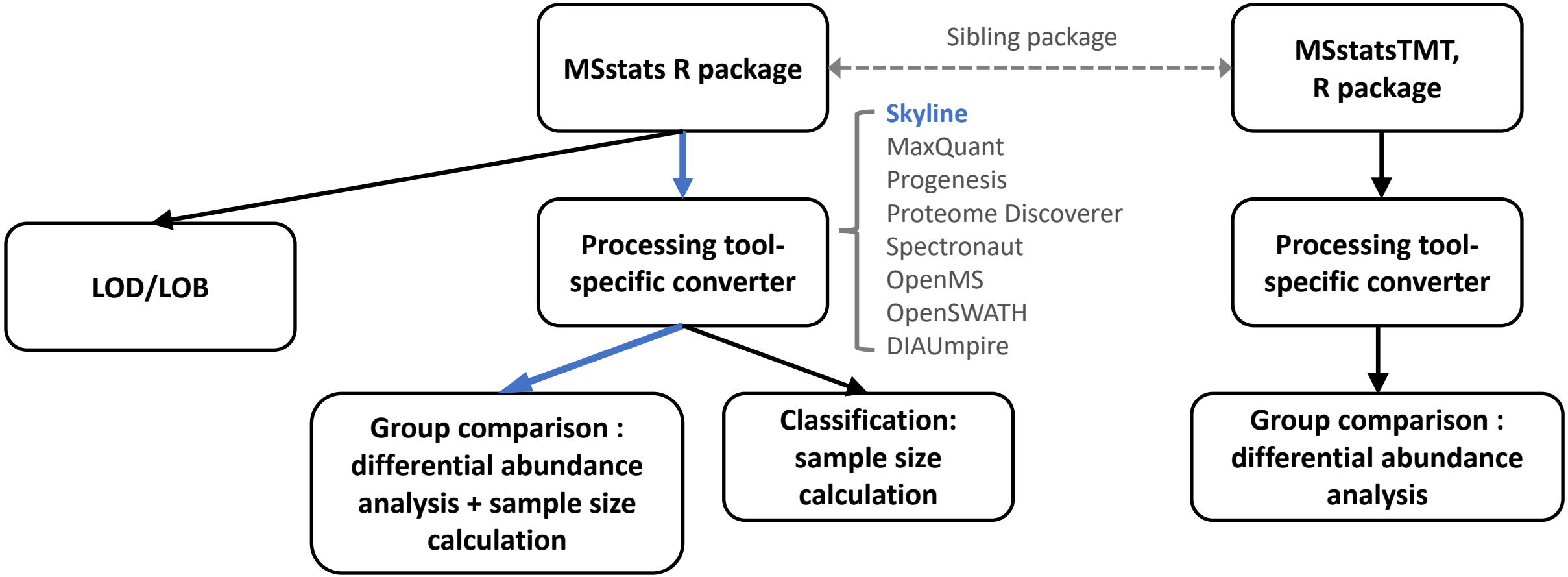
3. Different parameter settings have different results

N of significant proteins	Case1- iProphet cutoff=0.15	Case2- iProphet cutoff=0.05
Background protein	1	5
Spike protein	28 out of 30	28 out of 30





4. Other workflow with MSstats R package



* All materials for each workflow are available in msstats.org